REPORT ON THE STATUS OF AUTOMATIC AND CONTINUOUS WARNING EQUIPMENT AGAINST BIOLOGICAL WARFARE AGENTS IN THE AIR

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As an example, in an experiment with Thioflavine-T using a UG1/1mm exciter filter, yeast showed a violet fluorescence through RG1/2mm as secondary filter; by contrast talcum powder showed a green fluorescence but no visible or measurable motion with RG1/2mm as secondary filter. The differences in color reproduction of the various substances were striking and it is probable that one may be able to carry out an individual diagnostic differentiation of the various particle types (bacilli) by means of an analysis of the fluorescence spectrum.

In regard to the differentiation of sizes and brightness, we have thoroughly discussed the subject during the last visit but lacked the exact mathematical background for an evaluation. In the meanwhile, as is shown in the second part of this report, we have carried out an exact mathematical investigation with appropriate formulas and tables leading to unequivocal results, and enabling us to estimate the order of magnitude of the limits of tolerance of the procedure.

Very truly yours,

C A P GmbH

C. Schuck

REPORT ON THE FLUORESCENCE EXPERIMENTS

The purpose of this test series was to investigate and/or confirm the possibility of discriminating between organic and inorganic particles by means of different types of fluorescence phenomena. The particles to be investigated, however, were not placed on a microscopic slide in a dry state as is done in commercial fluorescence procedures but were led into our special chamber by means of water, that is, in a dissolved state, and were thus evaluated continuously.

Since the fluorescence of colorless organic particles is very weak and hence non-registrable, the organic and inorganic solutions under investigation were stained with fluorescent dyes. In this connection it was assumed that due to their differences in molecular structure and surface properties the organic and inorganic particles will exhibit different kinds of fluorescence phenomena in presence of equal amounts of fluorescent dyes and otherwise under the same conditions. This assumption was later confirmed in the experiments.

Yeast served as the organic substance and talcum powder as the inorganic substance. Both substances were dissolved in water which had been filtered several times. By observation of the appropriate sedimentation time it was possible to obtain particles of 5 to 20 μ maximal size. Both substances are commercially available in a relatively very high purity without difficulty. It is, however, much more significant that with talcum as the inorganic substance the result of the experiment is always on the more favorable side since talcum, in contrast with

yeast, hardly absorbs any light due to its bright white color, and hence reflects very intensive light impulses of all wave lengths. On the other hand when a yeast solution displays stronger light signals, these can only be interpreted as fluorescence.

The following fluorescent dyes were used in the test series: Euchrysine, Eosin, Diamond Phosphine, Coriphosphine-O, Thiazine Red, Rivanol, Thioflavine-S, Thioflavine-T, Acridine Yellow and Acridine Orange. Thioflavine-S and -T, and Acridine Yellow and -Orange were found to be much more suitable than the rest of the fluorescence media (see data-sheet extract). It was also found that the best results were obtained with fluorochrome dilutions of 1/5,000 to 1/10,000.

In order to obtain guiding values for the selection of filters, the pure fluorochromes and the experimental solutions of organic and inorganic particles stained with fluorochromes were exposed, by means of a rotatable glass prism, to monochromatic light of a wide variety of wave lengths (see data-sheet extract A and B).

In this manner we selected the filters BG12/1, UG11/4 and UG1/1 of Schott and Co. as excitation filters, and the filters RG1/2 and GG10/10 of the same firm as secondary filters.

In diagram 1 [see next page] the properties of the individual filters are compared. The values of the degree of pure transmission ϑ, or the ratio of the light current at the end of the passage through the filter to that at the beginning, is plotted against the wave length λ (nm). The scale of ϑ was chosen so that by vertical displacement of a given filter curve it is possible to obtain the characteristic curve of the same filter of higher or lower thickness.

One should make sure that the secondary filter has no fluorescence of its own. For this reason the above-mentioned filters were combined, in the beginning, in such a way that by placing the filters directly in front of each other a full absorption takes place, that is, the human eye cannot record any light effects. On the other hand on placing a fluorochrome between the filters color phenomena could be observed through the secondary filter. While theoretically this experimental arrangement appeared to be correct, in practice it was not realizable in a fully satisfactory manner since all excitation filters transmitted also light of longer wave lengths, even if with a lower intensity and hence invisibly (see Diagram 1). Hence a red-sensitive photomultiplier is not able to determine whether the light is primary light of longer wave lengths (over 700 nm) or fluorescent light.

This difficulty was solved by selecting a photomultiplier whose sensitivity range extends to approximately 650 nm and thus no longer responds to primary light of longer wave lengths. By a judicious selection of secondary filter it is possible to attain an overlapping of the sensitivity ranges of photomultiplier and secondary filter (see

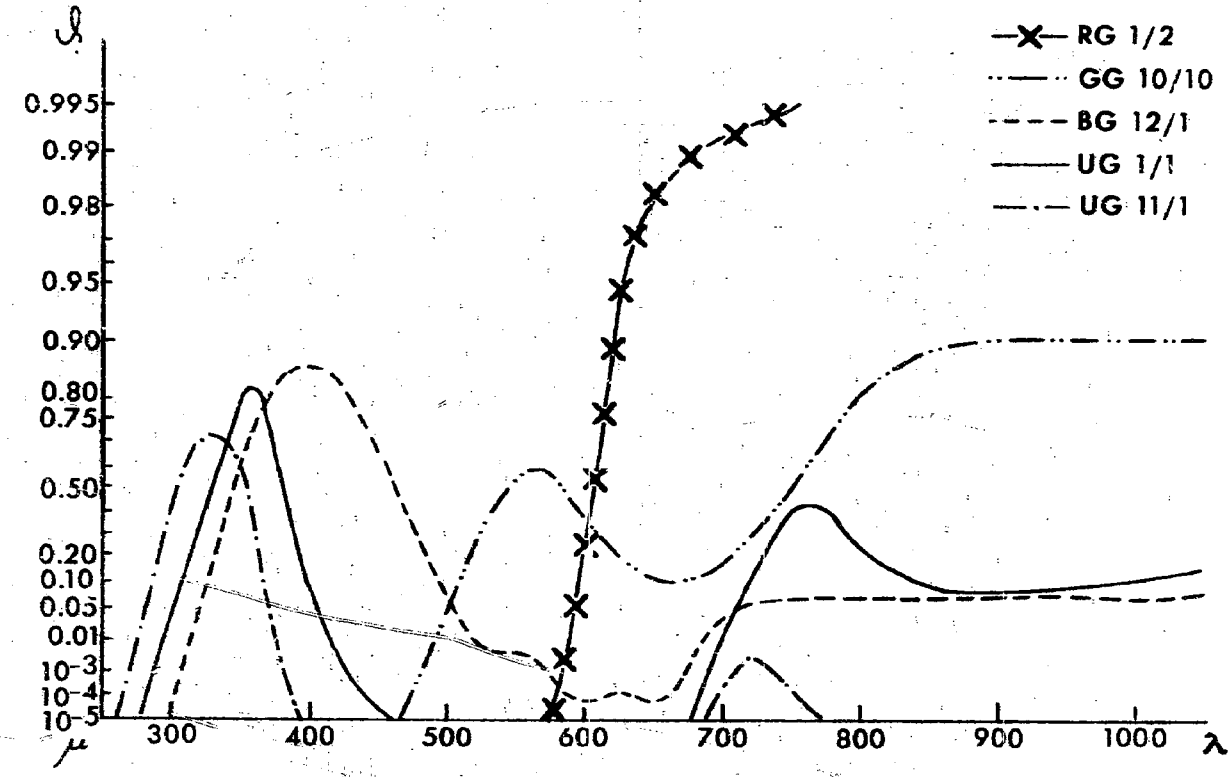
RG 1/2
GG 10/10
BG 12/1
UG 1/1
UG 11/1
0.995
0.99
0.98
0.95
0.90
0.80
0.75
0.50
0.20
0.10
0.05
0.01
10-3
10-4
10-5
300
400
500
600
700
800
900
1000
λ

Diagram 1

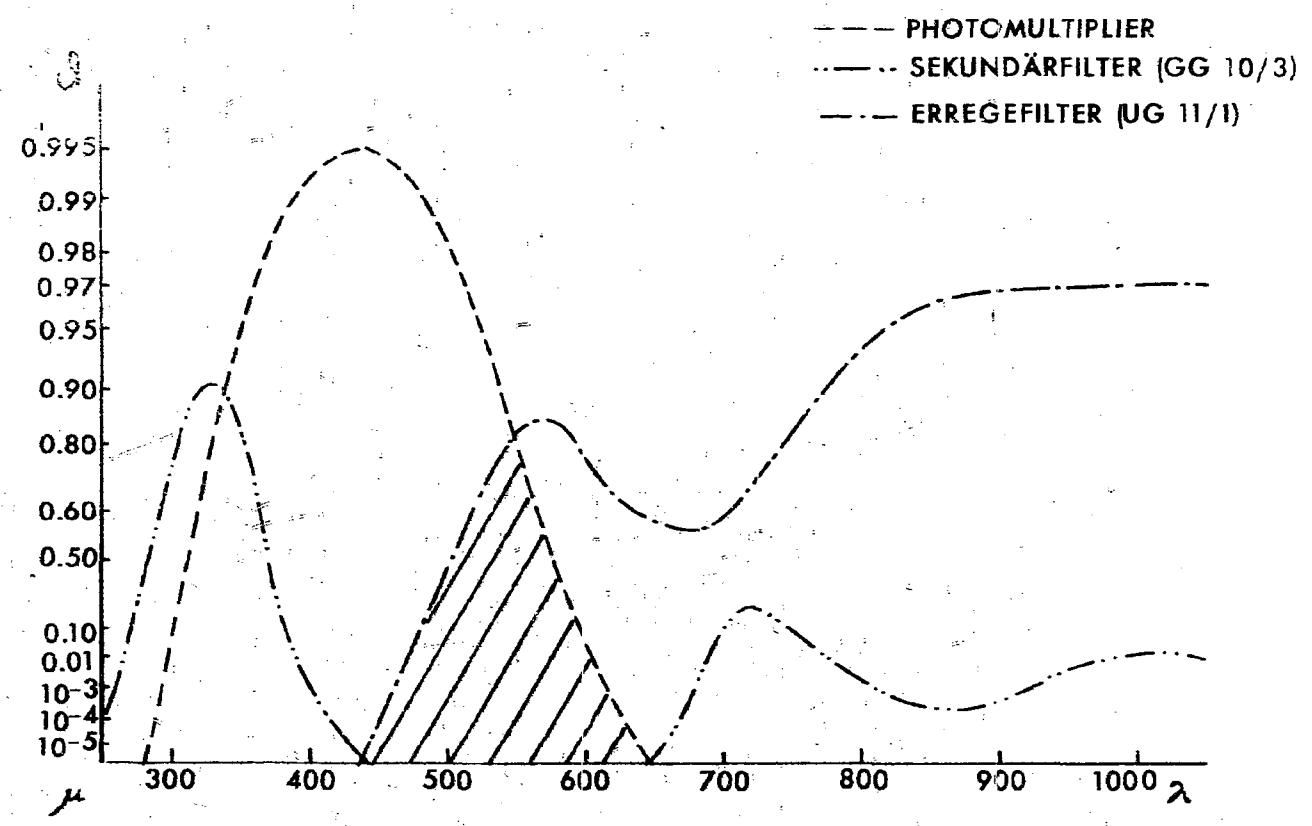

Diagram 2. 1) Excitation Filter 2) Secondary Filter

Diagram 2). In this way all impulses recorded by the photomultiplier are fluorescent light impulses.

The most suitable fluorochrome was found to be Acridine Orange, with which the difference in the intensities of the fluorescence impulses of organic and inorganic particles was the clearest and hence optimal. We employed a UG11/1 filter as primary filter and GG10/3 combined with RG1/1 as secondary filter. For light source we used a 150-watt xenon high-pressure lamp. The size of the particles varied between 5 µ and 20 µ.

It is quite likely that through the addition of suitable substances such as glycerol or sugar solution these fluorescence phenomena can be considerably intensified. This, however, did not appear to be necessary since even without it the impulse amplitudes of the organic particles were over 200 percent above those of the inorganic particles. Hence, through this method a process is indicated whereby one is able to differentiate between organic and inorganic particles.

Frankfurt - Main, 7 May 1962
Ho/ma

DATA-SHEET EXTRACT A

Fluorochrome	Dissolved Substance	Fluorescence Phenomenon up to Wave Length (nm)
Acridine Yellow	-	470
	Yeast	470
	Talcum	470
Acridine Orange	-	510
	Yeast	480
	Talcum	480
Thioflavine-S	-	-
	Yeast	400
	Talcum	400
Thioflavine-T	-	-
	Yeast	400
	Talcum	400
Rivanol	-	410
	Yeast	406
	Talcum	410
Thiosine Red	-	-
	Yeast	-
	Talcum	-
Coriphosphine-O	-	430
	Yeast	430
	Talcum	430
Diamond Phosphine	-	471
	Yeast	471
	Talcum	471
Eosin	-	520
	Yeast	500
	Talcum	400
Euchrysine	-	460
	Yeast	460
	Talcum	460

DATA-SHEET EXTRACT B

Fluorescence Medium	Excitation Filter	Yeast Fluorescence Color	Yeast Still Visible Through GG10/10	Yeast Still Visible Through RG1/2	Talcum Fluorescence Color	Talcum Still Visible Through GG10/10	Talcum Still Visible Through RG1/2	Remarks
Acridine Yellow	BG12/1	Lt. Green	x	x	Green	x	x	Yeast Stronger Impulse Than Talcum
	UG11/4	Green	x		"	x		
	UG1/1	"	x		"	x		
Acridine Orange	BG12/1	Green	x	x	Lt.Violet	x	x	"
	UG11/4	"	x		Pale Green	x		
	UG1/1	"	x		" "	x		
Thioflavine-S	BG12/1	Lt.Blue	x	x	Lt. Blue	x	x	"
	UG11/4	"	x		"	x		
	UG1/1	"	x		"	x		
Thioflavine- T	BG12/1	Lt. Blue	x	x	Lt.Blue	x	x	"
	UG11/4	--			Green			
	UG1/1	Violet	x	x	"	x		
Rivanol	BG12/4	Lt. Blue	x	x	Lt. Blue	x	x	"
	UG11/4	Green	x	x	Green	x	x	
	UG1/1	"	x	x	"	x	x	
Thiosine Red	BG12/4							
	UG11/4							
	UG1/1							
Coriphosphine-O	BG12/4	Lt. Blue	x	x	Lt.Green	x	x	"
	UG11/4	Green	x		Green	x		
	UG1/1	Violet	x	x	"	x		

DATA-SHEET EXTRACT B - Continued

Diamond Phosphine	BG12/4	Lt. Blue	x	x	Lt. Green	x	x	"
	UG11/4	Green	x		Green	x		
	UG1/1	"	x	x	"	x		
Eosine	BG12/4	Lt. Violet-Blue	x	x	Lt. Green	x	x	"
	UG11/4	Green	x		Green	x		
	UG1/1	Lt. Violet	x	x	"	x		
Fuchrysine	BG12/4	Lt. Blue	x	x	Lt. Green	x	x	"
	UG11/4	Green	x		Green	x		
	UG1/1	Lt. Blue	x	x	"	x		